permits a blank having no turbidity and therefore a very low absorbance. Furthermore, if unsatisfactory results are obtained due to accidental spillage or to an excessively high or low nitrate content, it is not necessary to repeat the entire incubation process, but merely the color development step using a suitable aliquot of supernatant solution. If many similar samples in the same range of nitrate content are to be studied, it appears that the procedure of Garner and coworkers could be employed advantageously. It employs a smaller amount of bacterial suspension to which the color reagent is directly added.

The time involved in determination of nitrate by the method described varies with the number of samples to be analyzed. A standard curve must be prepared for each batch of samples, although it may not be necessary to determine all the points indicated, as the Beer-Lambert law is followed between 2 and 20 γ of nitrate. With a large number of samples the actual time required for media preparation, centrifugation, etc., amounts to less than 20 minutes per sample.

Acknowledgment

The authors thank George B. Garner for many helpful suggestions and criticisms.

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Received for review August 11, 1958. Accepted December 19, 1958. Research supported by grants G-2150 National Science Foundation and the Texas Co. Journal paper No. 903, Nebraska Agricultural Experiment Station.

PLANT GROWTH REGULATORS

Synthesis and Biological Activity of Some Quaternary Ammonium and Related Compounds That Suppress Plant Growth

CHARLES F. KREWSON, JOHN W. WOOD,[1] and WINTHROP C. WOLFE[2]

Eastern Regional Research Laboratory, Eastern Utilization Research and Development Division, U. S. Department of Agriculture, Philadelphia 18, Pa.

JOHN W. MITCHELL and PAUL C. MARTH

Crops Research Division, U. S. Department of Agriculture, Beltsville, Md.

Certain quaternary ammonium and related compounds cause plants to grow short and sturdy, with intense green foliar coloration, and experimentally, they have prolonged the life of test plants, such as bean. The synthesis and chemistry of one of these compounds, Amo-1618, is described, as are the syntheses of structurally related compounds. Eight compounds were rated biologically as highly active; four were rated as moderately active, and nine were inactive inducing no biological response in young bean seedlings.

THE RELATIVELY HIGH DEGREE of potency of certain quaternary ammonium and other compounds (supplied by National Research Council, Chemical-Biological Coordination Center; synthesized by R. L. Shriner, State University of Iowa), which influence rate of growth, over-all size, and longevity of many kinds of plants, was discovered by Wirwillie and Mitchell (*14*), and the study was extended by Marth, Preston, and Mitchell (*9*). In a recent study using Amo-1618 on chrysanthemums, Cathey (*5*) found that, in addition to producing a retarding effect on growth, this compound was antagonistic to the growth accelerating effect of gibberellin.

The synthesis of one of the most important of the original compounds tested, Amo-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl 1-piperidinecarboxylate methyl chloride), and of additional new compounds, some of which also induce plants to develop short, sturdy stems with intense green foliar coloration, is presented. The corresponding iodide, designated Amo-1619, has previously been reported (*14*) as highly active. A compound, VIII, isomeric with Amo-1619, prepared from less expensive carvacrol, is also active. (See Table I for compound names and Figure 1 for structural formulas.)

[1] Present address, Laboratory of Chemical Pharmacology, National Cancer Institute, National Institutes of Health, Bethesda 14, Md.

[2] Present address, Organic Chemistry Section, Chemistry Division, National Bureau of Standards, Washington 25, D. C.

Experimental

Preparation of Amo-1618 (I) from Thymol. 4-Nitrosothymol. Prepared from thymol (reagent grade, 3.33*M*) according to the method of Kremers, Wakeman, and Hixon (*6*). Yield 83.1%, m.p. 159–161° C.; reported 87%, 160–164° C.

4-Aminothymol. Prepared from 4-nitrosothymol (*6*) using 3.50*M* quantities. Yield 93.0%; reported 82.1%.

4-Dimethylaminothymol Methiodide. 4-Aminothymol (528 grams, 3.19*M*) was added to anhydrous methanol

(2.5 liters). To this solution, during continuous stirring, was added anhydrous sodium carbonate (1240 grams, 11.70*M*) and then dropwise, under reflux, methyl iodide was added (1630 grams, 715 ml., 11.48*M*). The reaction was exothermal and refluxed spontaneously for about 15 minutes. Stirring was continued and a gentle reflux maintained for a 20-hour period. The cooled mixture was filtered with suction, the precipitate was washed with ether (1.5 liters) and suspended in water (6 liters). The stirred solution was acidified to congo red paper with concentrated hydrochloric acid (about 1.5 liters, 36% hydrochloric acid, specific gravity 1.18). The suspension was heated to boiling, diluted with sufficient hot water to completely dissolve the solids present and filtered with suction while hot. The chilled product was filtered off, and the light yellow precipitate was washed with ice water (about 5 liters). The product was dried to constant weight (yield 799 grams, 74.6%) at 60° C. under vacuum. The melting point was 232.0–232.5° C. (corr.).

Anal. Calcd. for $C_{13}H_{22}INO$: I, 37.86. Found, 37.90.

1-Piperidinecarbonyl Chloride (used as reagent). Benzene (400 ml., dried over sodium) was placed in a 2-liter, 3-necked flask, chilled in a dry ice ethyl alcohol bath, and the gross weight was recorded. During stirring with a Teflon-covered magnet, phosgene was bubbled into the benzene at 0° to 5° for about 20 minutes. Between the phosgene tank and the reaction flask, there was a mercury pressure regulator and a trap; the mercury regulator had an auxiliary exhaust tube connected to a hood drain. The discharge outlet of the flask was connected to a trap through a sodium hydroxide (20% solution) gas wash bottle; a discharge tube from this bottle went to the hood drain. The third neck of the flask was stoppered during this operation. The flask and its contents were weighed and by difference the weight of phosgene was 161 grams (1.63*M*). Dry piperidine (139 grams, 1.63*M*, or an equimolecular quantity to the amount of weighed phosgene was used) (dried over potassium hydroxide, redistilled, b.p. 105–106° C.) was dissolved in dry benzene (500 ml.). This solution was added dropwise with stirring to the benzene solution of phosgene at a temperature not exceeding 5°. A period of about 2½ to 3 hours was required. A total of 900 ml. of benzene was approximately the minimum quantity required to keep the temperature under proper control and to permit stirring.

The mixture was allowed to warm up to room temperature, and unreacted phosgene and benzene (about 300 ml.) were removed with the aid of heat and an aspirator. The mixture was cooled and the paste suction filtered through a coarse filter; the white solid (piperidine hydrochloride) was discarded. The filtrate and washings were combined and concentrated under partial vacuum to remove benzene. The product was distilled. A fore-run (16.0 grams, b.p. about 112° at 17.5 mm.) and a main fraction (120 grams, b.p. 111–113° at 16 mm.) were obtained (yield, approximately quantitative, 99.8%).

Twelve experiments were completed in order to improve operating conditions for the preparation of this compound. Variables were: concentration, temperature, rate, and techniques used, in the addition of phosgene to piperidine. Quantitative yields were obtained when the ratio of phosgene was 1*M*, or slightly less but not greater than 1*M*, to 1*M* of piperidine. A quantitative yield is a 50% figure as piperidine is utilized in the formation of piperidine hydrochloride from hydrogen chloride, a reaction product. When a molecular ratio of 2 piperidine (and higher) to 1 phosgene was used, a poor yield of 1-piperidine carbonyl chloride was obtained; in addition to piperidine hydrochloride, a large amount of *N,N,N',N'*-bispentamethyleneurea was formed:

Table I. Results of Standard Lanolin-Bean Screening Tests on Quaternary Ammonium Carbamates and Related Compounds

	Compounds	Activity, %[a] Terminal Growth Weight[b]	Terminal Growth Length[c]	First internode length[d]
	HIGHLY ACTIVE[e]			
I	2-Isopropyl-4-dimethylamino-5-methylphenyl 1-piperidinecarboxylate methyl chloride	−87	−94	−80
II[f]	(2-Hydroxy-3-cyclohexylbenzyl)methylpiperidinium bromide dimethylcarbamate	−66	−88	−46
III	2-*tert*-Butyl-4-dimethylamino-5-methylphenyl 1-piperidinecarboxylate methiodide	−62	−85	−65
IV[f]	(2-Hydroxy-3-cyclohexylbenzyl)trimethylammonium bromide dimethylcarbamate	−63	−83	−35
V	2-*tert*-Butyl-4-dimethylamino-5-methylphenyl 1-piperidinecarboxylate methyl chloride	−71	−82	−57
VI	2-Isopropyl-4-dimethylamino-5-methylphenyl-*N,N*-dimethylcarbamate methiodide	−65	−81	−53
VII[f]	(2-Hydroxy-5-phenylmercaptobenzyl)trimethylammonium bromide dimethylcarbamate	−52	−78	−49
VIII	3-Isopropyl-4-dimethylamino-6-methylphenyl 1-piperidinecarboxylate methiodide	−65	−74	−40
	MODERATELY ACTIVE[e]			
IX	2-Isopropyl-4-dimethylamino-5-methylphenyl-*N,N*-diethylcarbamate methiodide	−37	−66	−34
X[f]	3-Hydroxyphenyltrimethylammonium methylsulfate dimethylcarbamate (Prostigmine methylsulfate)	−34	−53	−27
XI	4-Dimethylaminophenyl 1-piperidinecarboxylate methiodide	−22	−37	−57
XII	2-Isopropyl-4-nitroso-5-methylphenyl 1-piperidinecarboxylate	−13	−36	−30
	INACTIVE[e]			
XIII	2-Isopropyl-4-dimethylamino-5-methylphenyl 1-piperidinecarboxylate hydrochloride	−25	−21	−5
XIV	4-Aminothymyl 1-piperidinecarboxylate hydrochloride	−13	−20	0
XV	3-Dimethylaminophenyl 1-piperidinecarboxylate methiodide	−10	−20	−18
XVI	2-Isopropyl-4-(1-piperidinecarboxamido)-5-methylphenyl 1-piperidinecarboxylate	−12	−16	−5
XVII	2-Isopropyl-5-methylphenyl 1-piperidinecarboxylate (thymyl 1-piperidinecarboxylate)	−7	−16	−22
XVIII	4-Dimethylaminothymylbenzoate methiodide	+1	−2	−17
XIX	3-Dimethylaminophenyl-*N,N*-dimethylcarbamate methiodide	+4	+1	−25
XX	4-Dimethylaminothymylacetate methiodide	+8	+2	−7
XXI	2-Isopropyl-4-chloro-5-methylphenyl 1-piperidinecarboxylate	+23	+8	−11

[a] Subtract average measurement of treated plants from that of comparable untreated ones, divide difference by average measurement of untreated plants, and multiply by 100.

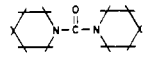
[b] Increase or decrease in weight of terminal growth (all growth above 2nd node) which developed after treatment compared with that which developed on comparable untreated plants.

[c] Increase or decrease in length of growth above 2nd node which developed after treatment compared with that which developed on comparable untreated plants.

[d] Increase or decrease in length of 1st internode which developed after treatment compared with that which developed on comparable untreated plants.

[e] Compounds having activity between 71 and 100%, 21 and 70%, or 0 and 20% are arbitrarily classified as highly active, moderately active, or inactive, respectively.

[f] Supplied by J. A. Aeschlimann of Hoffmann-LaRoche, Inc. (*3,4,7*).



This compound was tested in the same manner as compounds I-XXI (Table I) and was inactive. An alternate five-step method (*13*), starting with ethyl oxalate and piperidine, gave a very poor over-all yield in this laboratory.

2 - Isopropyl - 4 - dimethylamino - 5-methylphenyl 1- Piperidinecarboxylate Methiodide (Amo-1619). A mixture of 4 - dimethylaminothymol methiodide (225 grams, 0.671*M*), chloroform (1.2 liters dried over calcium chloride and distilled from phosphorus pentoxide), pyridine (600 ml., dried over sodium hydroxide and calcium oxide, freshly distilled, b.p. 114–115° C.) and 1-piperidinecarbonyl chloride (200 grams, 1.36), quantities greater than this amount did not increase the yield) was shaken in a 1-gallon stoppered bottle for 16 hours. The clear orange solution was poured into 4 liters of anhydrous ether, the mixture was triturated and allowed to stand for the solid to settle. The ether was decanted, and the product (Amo-1619) was washed twice with ether (750-ml. and 200-ml. portions). The Amo-1619 was recrystallized from 2 liters of boiling water and suction-filtered while hot. The product was removed from the chilled filtrate by suction, washed with ice water (about 250 ml.) and dried in a vacuum desiccator. Yield of light tan crystals was 256 grams, 86.4% yield, m.p. 169–174° C. (corr.); white crystals (m.p. 182.3–183.3° C., corr., dec.) were obtained by a second recrystallization from hot water (90° C.). Over-all yield from thymol (reagent grade), 49.8%.

Anal. Calcd. for $C_{19}H_{31}IN_2O_2$: I, 28.43 N, 6.28. Found: I, 28.57; N, 6.28.

Amo-1618 (I). To freshly prepared silver chloride (85.0 grams), which had been thoroughly washed with water, was added Amo-1619 (89 grams), distilled water (500 ml.), and ethyl alcohol (500 ml. of 95%). This mixture was shaken for 20 hours. The mixed silver halides were removed on a fine filter, and the halide residue was washed twice with 100-ml. portions of 95% ethyl alcohol. The solvent was removed from the combined filtrate and washings, and the product obtained was a white amorphous solid [weight 65.5 grams, 92.9% yield, m.p. 150.5–151.5° C. (corr.)]. Recrystallized from absolute methanol and precipitated with anhydrous ether, the Amo-1618 (I) melted at 151–152° C. (corr.).

Anal. Calcd. for $C_{19}H_{31}ClN_2O_2$: Cl, 9.99. Found: Cl, 9.28.

The above six-step process, beginning with thymol, is pictured in Figure 2.

Preparation of Compound III from 6-*tert*-Butyl-3-cresol. 2-*tert*-Butyl-5-methyl-4-nitrosophenol. The technique used was similar to that described for the preparation of 4-nitrosothymol. 6-*tert*-Butyl-3-cresol (100 grams, 0.610*M*, tech. grade) was used with sodium nitrate (72 grams, 1.04*M*). A dry ice alcohol bath was used to maintain a working temperature of −5 to 0°. The addition of nitrite required about 15 minutes. The reaction mixture was poured into water (8 liters). An oil separated which solidified on standing. The yield of crude product was 111.5 grams [94.7%, m.p. 155–159° C. (uncorr.).]. The crude product recrystallized from benzene (2 liters), gave 67 grams of sharply melting yellow needles (180° C., corr.).

4-Amino-5-*tert*-butyl-5-methylphenol. The procedure used was similar to that used above for 4-aminothymol. 2-*tert*-Butyl - 5 - methyl - 4 - nitrosophenol (66 grams, 0.34*M*) was dissolved in a dilute ammonia solution (1.4 liters of 28% aqueous ammonia and 1.0 liter of water). Warming was necessary to completely dissolve the phenol. Yield of crude product dried under vacuum to constant weight was 55.5 grams [90.7%, m.p. 180–182° C. (corr.)].

2 - tert - Butyl - 4 - dimethylamino - 5-methylphenol Methiodide. This compound's preparation follows the procedure used for 4-dimethylaminothymol methiodide. 4-Amino-2-*tert*-butyl-5-methylphenol (55 grams, 0.31*M*), anhydrous sodium carbonate (117 grams, 1.11*M*), anhydrous methanol (250 ml.), and methyl iodide (a total of 237 grams, 105 ml., 1.66*M*) were used. The final crude product weighed 74 grams (69%, m.p. 206.5–207.5° C.), which upon recrystallization from boiling water (1 liter) gave 69 grams (64%) of white needles melting sharply at 206.5° C. (corr.).

2 - *tert* - Butyl - 4 - dimethylamino-5-methylphenyl 1-Piperidinecarboxylate Methiodide (III). This compound was prepared by the same method as that described above for Amo-1619. 2-*tert*-Butyl-4-dimethylamino-5-methylphenol methiodide (10 grams, 0.029*M*), 1-piperidinecarbonyl chloride (12.7 grams, 0.0858*M*), dry piperidine (25 ml.), and dry chloroform (50 ml.) were used. An orange oil separated when the reaction mixture was poured into dry ether (400 ml.). The ether was decanted and the oil was triturated three times with small amounts of ether. Water (50 ml.) was added to pasty residue which did not solidify. This mixture was heated to boiling to effect solution, additional hot water (20 ml.) was required to bring the product into solution. The product crystallized upon refrigera-

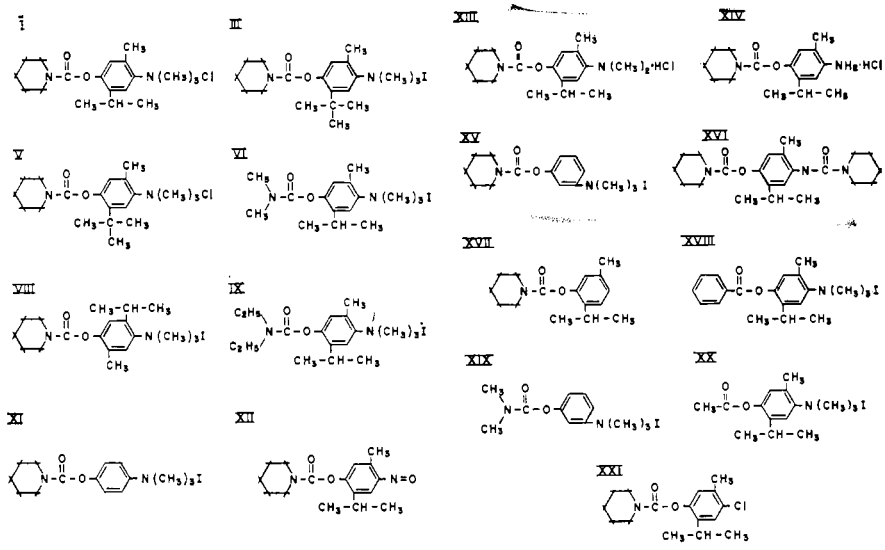


Figure 1. Structural formulas of the compounds used in this study and listed in Table I

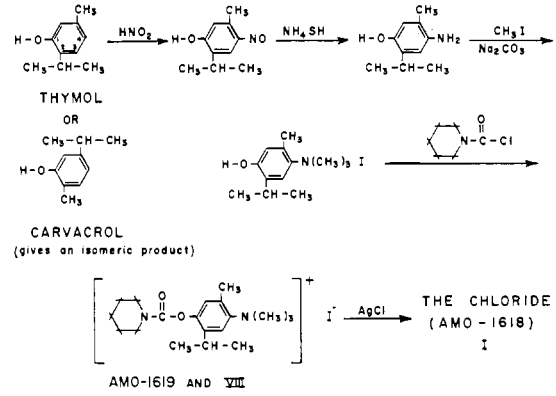


Figure 2. A six-step process for the preparation of Amo-1618 from terpene thymol

tion. Yield of dried product, III, (20 hours, vacuum oven at 50° C.) 9.69 grams (75.1%), m.p. 184.5–186.5° C. (corr., sintered at 182.5° C.).

Anal. Calcd. for $C_{20}H_{33}IN_2O_2$: I, 36.34; N, 4.01. Found: I, 34.55; N, 3.66.

Preparation of Compound V from the Methiodide (III). This chloride was prepared by conversion of the iodide as previously described for the preparation of Amo-1618 from Amo-1619. The methiodide (4.6 grams, 0.010*M*) with appropriate quantities of reagents produced 3.1 grams (83.9% yield) of product, V, m.p. 153.4–156.7° C. (corr.).

Anal. Calcd. for $C_{20}H_{33}ClN_2O_2$: N, 7.58. Found: N, 6.56.

Preparation of Compound VI from Dimethylamine. Dimethylamine. Dimethylamine hydrochloride (100 grams, 1.22*M*) was added dropwise to a warm solution—heated on steam bath—of sodium hydroxide (200 grams in 200 ml. of water) with stirring in a generator flask. The gas evolved was dried through a sodalime drying tower and absorbed in a tared dry ice chilled receiver containing benzene (250 ml.). The amount of dimethylamine absorbed was 50 grams, 1.1*M* (90% recovery).

N,N - Dimethylcarbamyl Chloride. This compound was prepared by similar techniques to those used for 1-piperidinecarbonyl chloride. To phosgene (120 grams, 1.27*M*) absorbed in dry benzene (400 ml.) was added dropwise dimethylamine (50 grams, 1.1*M*), dissolved in 250 ml. of dry benzene. The product—colorless liquid—distilled at 161–5° C. Reported (*7*) 167° C. Yield 55.0 grams (93.2%).

Compound (VI). Its preparation was similar to 2-isopropyl-4-dimethylamino-5-methylphenyl 1-piperidinecarboxylate methiodide described above. 4 - Dimethylaminothymol methiodide (13.4 grams, 0.0400*M*), *N,N*-dimethylcarbamyl chloride (8.6 grams, 0.080*M*), dry chloroform (75 ml., ethyl alcohol-free), and dry pyridine (35 ml.) were shaken 16 hours. Additional *N,N*-dimethylcarbamyl chloride (8.6 grams, 0.080*M*) was added and shaking continued another 16 hours. Yield of VI 8.4 grams (51.8%), m.p. 176–7° C., corr.

Anal. Calcd. for $C_{16}H_{27}IN_2O_2$: N, 6.90. Found: N, 6.92.

Preparation of Compound VIII from Carvacrol. 4-Nitrosocarvacrol. Prepared by method used for 4-nitrosothymol. Carvacrol (100 grams, 0.666*M*, redistilled, b.p. 234–7° C., uncorr.), ethyl alcohol (500 ml., 95%), hydrochloric acid (500 ml., 36%), and sodium nitrite (72 grams, 1.0*M*) were used. Yield of crude product 108 grams (90.6%, used to calculate over-all yield of compound VIII), m.p. 143–7°C., corr. Recrystallized from boiling benzene (1.5 liters) and washed with benzene the yield was 89.0 grams (74.6%) m.p. 147–150° C. (corr.). After a second recrystallization from benzene the yield was 75.0 grams (62.8%), m.p. 153–4° C. (corr.).

4-Aminocarvacrol. Prepared similarly to 4-aminothymol. 4-Nitrosocarvacrol (70 grams, 0.39*M*) and dilute ammonia solution (690 ml. of 28% aqueous ammonia in 1.1 liters of water) were used. Yield 59 grams (91%), m.p. 129.5–131.5° C. (corr.). Reported m.p. 134° C. (*10*).

4-Dimethylaminocarvacrol Methiodide. Similar to 4-dimethylaminothymol methiodide. 4-Aminocarvacrol (55.0 grams, 0.333*M*), anhydrous methanol (260 ml.), anhydrous sodium carbonate (130 grams, 1.23*M*), and methyl iodide (170.9 grams, 75 ml., 1.200*M*) were used. After an 18.5-hour reflux, additional methyl iodide (25 ml.) was added, and refluxing was continued another 6.5 hours. Yield 77 grams (69% used to calculate over-all yield of compound VIII) m.p. 206.5–209.5° C., corr. Recrystallized from hot water (600 ml.) gave 67.0 grams (60.0% yield), m.p. 207–209° C., corr.

Compound VIII. Prepared in similar fashion to Amo-1619 the corresponding thymol isomer described above. 4-Dimethylaminocarvacrol methiodide (33.5 grams, 0.100*M*) dry pyridine (90 ml.) and 1-piperidinecarbonyl chloride (30 grams, 0.20*M*) were shaken for 16 hours. Additional 1-piperidinecarbonyl chloride (30 grams) was added because undissolved solids were present. After 15 hours of additional shaking, the reaction mixture was a clear red-orange solution. Yield 32.0 grams (71.8%, used to calculate over-all yield of compound VIII), m.p. 170.5–175.5° C., corr. Recrystallized from hot water (300 ml.), yield was 27.8 grams (62.4%), m.p. 172.5–175.0° C., corr. Further recrystallization from hot water (250 ml.) gave 24.8 grams (55.7%), m.p. 171.5–172.5° C., corr. Over-all yield from carvacrol 34.4%.

Anal. Calcd. for $C_{19}H_{31}IN_2O_2$: I, 28.43; N, 6.28. Found: I, 28.45; N, 6.33.

Preparation of Compound IX from Diethylamine. *N,N*-Diethylcarbamyl Chloride. Preparation similar to *N,N*-dimethylcarbamyl chloride. Diethylamine (140 grams, 1.92*M* from technical grade over sodium hydroxide, freshly distilled, b.p. 55–55.5° C.) and phosgene (190 grams in 400 ml. of dry benzene) were used. Diethylamine recovered as hydrochloride, 77 grams. Product distilled at 184–186° C., yield 124 grams (95.6%).

Compound IX. A mixture of *N,N*-diethylcarbamyl chloride (10.9 grams, 0.0803*M*), 4 - dimethylaminothymol methiodide (13.4 grams, 0.0400*M*) dry chloroform (75 ml.), and dry pyridine (35 ml.) was shaken for 16 hours. Because of undissolved reactant, additional *N,N* - diethylcarbamyl chloride (10.9 grams, 0.0803*M*) was added, and the mixture was shaken for another 16 hours. The product, IX, recrystallized twice from boiling water, melts at 162.5–169.5° C., uncorr. weight 5.00 grams (28.8% yield).

Anal. Calcd. for $C_{18}H_{31}IN_2O_2$: C, 49.77; H, 7.19; N, 6.45. Found: C, 49.00; H, 6.88; N, 5.92.

Preparation of Compound XI from 4-Aminophenol Hydrochloride. 4-Dimethylaminophenol Methiodide. 4-Aminophenol hydrochloride (29 grams, 0.20*M*, purified) was dissolved in methanol (200 ml. C.P.) anhydrous sodium carbonate (106 grams, 1.01*M*) added and methyl iodide (114 grams, 50 ml., 0.790*M*) added dropwise under reflux during continuous stirring; reaction mixture refluxed spontaneously for about $^1/_2$ hour following which it was refluxed by heating for 18 hours. An additional quantity of methyl iodide (25 ml.) was added and reflux continued for another 8 hours. The product was filtered off from the cooled mixture, suspended in water (400 ml.) and acidified to congo red with concentrated hydrochloric acid (100 ml.). The solid was suction filtered, washed, and dried in vacuo for 72 hours. Weight 12.0 grams (21.5%); m.p. 217.5–219.5° C. Recrystallized from boiling water (100 ml.) gave 9.5 grams (yield 17%), m.p. 225.5–226.5° C. (sealed capillary; m.p. bath preheated to 210° C.).

Compound XI. Prepared in the manner described for Amo-1619. 4-Dimethylaminophenol methiodide (8.4 grams, 0.031*M*), dry chloroform (60 ml.), dry piperidine (60 ml.), and 1-piperidinecarbonyl chloride (9.0 grams 0.061*M*) were used. After the mixture was shaken for 16 hours, additional 1 - piperidinecarbonyl chloride (9.0 grams) was added and the shaking continued another 15 hours. Yield of crude product, XI, was 13.5 grams, m.p. 177–190° C. Recrystallized from boiling absolute ethyl alcohol (100 ml.) gave 7.4 grams (63% yield) of product melting at 174–5° C. (corr. dec., m.p. bath preheated to 167° C.). Repeated recrystallizations appear to cause decomposition as evidenced by melting point ranges 172–182°, 184–208°, and 174–188° C.

Preparation of Compound XII from 4-Nitrosothymo'. 4-Nitrosothymol (24 grams, 0.13*M*) was dissolved in a solution of dry pyridine (120 ml.) and dry chloroform (240 ml.). To this was added 1-piperidinecarbonyl chloride (38 grams, 0.26*M*), the reactants were thoroughly shaken and allowed to stand 17 hours. The orange solution was triturated and thoroughly washed by decantation with portions of anhydrous ether as described above for the preparation of Amo-1619.

The main reaction product, 2-isopropyl-4-nitroso-5-methylphenyl 1-piperidinecarboxylate, was soluble in ether in contrast to the compound prepared from 4 - dimethylaminothymol methiodide. The ether extracts were therefore combined, evaporated to about 150 ml. and washed three times with ice water (200, 50, and 50 ml.). The ether was removed, and the product was vacuum dried (1 hour at 40°). On cooling, after standing 20 hours in a vacuum desiccator, the product partially crystallized. It was thoroughly washed by slurring three times with *n*-hexane (150, 100, and 50 ml.). Yield of dried product (XII), 20 grams (69%), m.p. 107–108° C., uncorr.; (not raised by additional recrystallizations).

Anal. Calcd. for $C_{16}H_{22}N_2O_3$: C, 66.18; H, 7.64. Found: C, 67.25; H, 7.71.

Method of Application of Chemicals to Bean Plants. The test compound (12.5 mg.) was dissolved in or mixed with 0.25 ml. of Tween 20 contained in a small vial; 1 gram of melted lanolin was then added and stirred until cool, forming a creamy paste mixture. Approximately 12 mg. of this mixture was applied as a band 3 to 5 mm. wide and about 1 mm. thick around the first internode of five young bean plants of the Black Valentine variety. At the time of treatment the plants had developed primary leaves that were about 3 cm. wide, and the trifoliate leaves were still tightly folded in the terminal buds. The treated plants were grown for a period of 7 to 9 days, and measurements of the first internode and terminal growth were recorded and compared with untreated plants (Table I). Responses that developed during later stages in the maturation of the plants were not studied in these tests.

Results and Discussion

Eleven of the new compounds tested possessed moderate to high activity and nine were relatively ineffective (Table I). The active compounds greatly reduced elongation of the first internodes and terminal growth of the test plants during the experiment. Leaves of plants treated with these substances were somewhat deeper green in color than were those on untreated ones.

In addition to the inactive compounds listed in Table I, the following were also inactive:

INTERMEDIATE OR RELATED COMPOUNDS

Thymol	4-Dimethylaminothymol methiodide
4-Chlorothymol	*N*,*N*-Diethyloxamic acid
4-Aminothymol	Ethyl-*N*-piperidyloxamate
4-Nitrosothymol	*N*-piperidyloxamic acid
4-Dimethylaminomethylthymol	

COMMERCIALLY SUPPLIED COMPOUNDS

(2-Hydroxy-5-phenylbenzyl)trimethylammonium bromide dimethylcarbamate
(2-Hydroxy-5-phenylbenzyl)dimethylammonium chloride dimethylcarbamate
(2-Hydroxy-5-cyclohexylbenzyl)trimethylammonium bromide dimethylcarbamate
(2-Hydroxy-5-phenylbenzyl)-1-methylpiperidinium bromide dimethylcarbamate
(2-Hydroxy-5-phenoxybenzyl)trimethylammonium bromide dimethylcarbamate

A number of variations in chemical structure, centering around one of the most active compounds, 2-isopropyl-4-dimethylamino - 5 - methylphenyl 1-piperidinecarboxylate methyl chloride (I) referred to in previous publications (*9*, *14*) as (4-hydroxy-5-isopropyl-2-methyl)trimethyl ammonium chloride, 1-piperidinecarboxylate (Amo-1618), have been made in an attempt to correlate biological activity with structural configuration. Although deductions from these experiments are not clear-cut certain tendencies are discussed briefly in the interest of future investigations along these lines.

The active quaternary compound Amo-1618 appears to consist of the inactive tertiary amine, XIII quaternized by methyl substitution for hydrogen. This produces a positive ionic-type bond, a hydrophilic portion, which may be an important contributing factor to the activity of this type of compound.

Compounds of this series possessing the growth retarding properties contain two nitrogen atoms to the molecule, one a quaternary, one a carbamate nitrogen. Possibly the removal of either inactivates the molecule. The inactive compound, XVII, containing only the carbamate nitrogen, best illustrates this point. The inactivity of compounds XII, XIII, XIV, XVI, and XXI, further illustrates this point although XII did show some slight activity.

Absence of carbamate nitrogen may render Amo-1618–type compounds inactive as shown by the lack of growth retarding effects of 4-dimethylaminothymol methiodide, XVIII, and XX.

The nonnecessity, but seeming enhancement, of activity by substitution of methyl, isopropyl or tertiary butyl on the aromatic ring suggests an increased effect from an optimum molecular size approaching that of Amo-1618. Ring substitution possibly exerts a counter influence on the hydrophylic quaternary nitrogen and the potentially available hydrophylic phenolic group arising from the carbamate portion of the molecule. On the purely speculative side, the function of the carbamate group may be to shield or alter the interphase distribution of a phenolic group as an aid to its entrance into plant tissues; after ingress, a systemic distribution of liberated phenol could exert a generalized effect in regulating plant growth.

Positions of the quaternary group of an α-toluyl group in three cases of activity, II, IV, and VII, indicate that it is not necessary for the quaternary nitrogen to be directly attached to an aromatic nucleus.

Although in this series compounds presenting ortho and para ring substitutions are few in number, they serve as an indication that activity may be greater where a carbamate aromatic oxygen appears para to the quaternary nitrogen. The influence may be less for ortho and least for meta substitution.

Acknowledgment

The authors are indebted to T. D. Fontaine, National Science Foundation (formerly of the Eastern Regional Research Laboratory) for his interest and assistance, to J. S. Ard for analytical data and for his criticisms in this problem, and to C. H. H. Neufeld, Western Regional Research Laboratory (formerly of the Eastern Regional Research Laboratory) whose synthesis of Amo-1618, with some modifications, checked their own.

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Received for review September 4, 1958. Accepted December 18, 1958. Presented in part before Division of Agricultural and Food Chemistry, 134th Meeting, ACS, Chicago, Ill., September 1958. Mention of Hoffman-La Roche Inc., Roche Park, N. J., does not imply endorsement by the U. S. Department of Agriculture over companies not mentioned.